

HUMAN T CELL  $\gamma$  GENES ARE FREQUENTLY  
REARRANGED IN B-LINEAGE ACUTE LYMPHOBLASTIC  
LEUKEMIAS BUT NOT IN CHRONIC  
B CELL PROLIFERATIONS

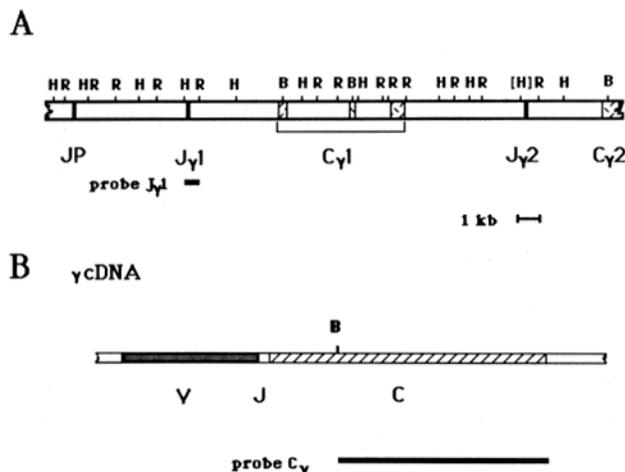
BY ZHU CHEN,\* DENIS LE PASLIER,<sup>‡</sup> JEAN DAUSSET,<sup>‡</sup> LAURENT DEGOS,<sup>‡</sup>  
GEORGES FLANDRIN,\* DANIEL COHEN,<sup>‡</sup> AND FRANCOIS SIGAUX\*

*From the \*Institut National de la Santé et de la Recherche Médicale U301 and Laboratoire  
Central d'Hématologie; and <sup>‡</sup>INSERM U93, Hôpital Saint Louis,  
75475 Paris, Cedex 10, France*

Rearrangements of genes encoding the Igs and the T cell antigen receptor (TCR)<sup>1</sup> are considered as useful markers for determining clonality and B or T cell line involvement in lymphoid malignancies (1–4). It has become apparent, however, that these rearrangements are not entirely specific for a given cell line, and a number of cases of Ig gene rearrangements have been documented in T cell proliferations (4, 5). More recently, rearrangement of the genes of the TCR $\beta$  chain has been found particularly in so-called non-T, non-B acute lymphoblastic leukemia (ALL) (6, 7), which is currently considered to be a clonal proliferation of cells with the same phenotype as normal B cell precursors (8). Gene rearrangements were also found in some cases of acute myeloid leukemia (AML) (9–12). The significance of these rearrangements remains unclear, but it is interesting to note that they seem to occur only in genes whose rearrangement takes place in the earliest phase of lymphoid maturation. Thus to date, in typical T cell proliferations, only rearrangement of the H chains of Igs, which precedes that of the  $\kappa$  and  $\lambda$  chains (13), has been described. Correspondingly, TCR $\beta$  gene rearrangement precedes TCR $\alpha$  gene rearrangement during thymic maturation (14), and currently only TCR $\beta$  gene rearrangement has been detected in B cell malignancies. These findings suggest that these rearrangements may occur in very immature cells at a stage when they still have both T and B potentiality. Beside the TCR $\beta$  and  $\alpha$  genes that encode the T<sub>i</sub> proteins, a third gene termed T cell rearranging gene  $\gamma$  (TRG $\gamma$ ) or T cell  $\gamma$  chain gene, undergoes somatic rearrangements in T cell (15, 16). The organization of the TRG $\gamma$  is largely elucidated. 9 (17) to 12 (18) variable segments (V $\gamma$ ), including at least five pseudogenes, are located upstream of two strongly homologous regions, each comprising a junctional segment (J $\gamma$ ) and a constant region (C $\gamma$ ) (16, 19). The

Z. Chen is the recipient of a fellowship from the Fondation contre la Leucémie. Address correspondence to F. Sigaux, INSERM U301, Hôpital Saint Louis, 2 place du Docteur Fournier, 75475 Paris, Cedex 10, France.

<sup>1</sup> *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; HCL, hairy cell leukemia; TCR, T cell antigen receptor; TRG $\gamma$ , T cell rearranging gene  $\gamma$ .



**FIGURE 1.** (A) Organization of the  $J\gamma$  and  $C\gamma$  regions. This restriction map is adapted from references 16–18; the relevant Hind III (*H*), Eco RI (*E*), and Bam HI (*B*) sites are indicated; not all restriction enzyme sites are shown. (*JP*) the joining region defined in reference 17. The polymorphic Hind III site is indicated in parentheses. (B) Schematic representation of the PHT  $\gamma$ -9 cDNA. The  $C\gamma$  probe is a 0.7 kb Bam HI fragment corresponding to a part of exon 2 and all of exon 3.

$V\gamma$  genes can recombine with both regions  $J\gamma 1$  and  $J\gamma 2$  and also with a segment called  $J\gamma P$  (17) that is located at about 4 kb in 5' of the  $J\gamma 1$  region (Fig. 1). Since  $TRG\gamma$  rearrangement probably precedes  $TCR\beta$  gene rearrangement and can thus be seen very early in thymic maturation (20), it would appear conceivable that, analogous to the  $TCR\beta$  genes, the  $TRG\gamma$  are frequently rearranged in B-lineage ALL. Whereas the human  $TRG\gamma$  structure has been extensively studied, the distribution of the rearrangements in the different cell lines has not been extensively studied. In the present study, by using a genomic probe that recognizes segments  $J\gamma 1$  and  $J\gamma 2$ , we have shown the absence of  $TRG\gamma$  rearrangement in 9 cases of typical acute myeloid leukemia and 19 chronic B cell lymphoid proliferations. On the other hand,  $TRG\gamma$  rearrangement was noted in 18 of 22 B-lineage ALL, whereas  $TCR\beta$  gene rearrangement was present in only 9 of the cases. We have also shown that  $TRG\gamma$  rearrangement most often involves the  $J\gamma 1$  region on a single allele and thus differs from what is generally seen in T cell ALL also studied in this paper and in which the rearrangement generally involves the  $J\gamma 2$  regions of both alleles. Finally, in only 2 of the 13 cases of B-lineage ALL for which the  $TRG\gamma$  mRNA expression was studied, 1.6-kb transcripts were seen, but the expression was very weak. No  $TCR\beta$  mRNA transcription was detected in the 13 cases analyzed.

### Materials and Methods

**Cells.** The malignant cells were separated on a Ficoll gradient and then used as is and/or after conservation in liquid nitrogen. Morphological and immunological characterization was based on the usual criteria (8, 21, 22). The surface phenotype of the malignant cells was determined by a standard method of indirect immunofluorescence and cytofluorograph reading (model 50 H; Ortho Diagnostic Systems Inc., Westwood, MA) using a large panel of mAbs that recognize T-associated or T-restricted antigens (anti-CD1 to CD5, CD7, CD8) and B-restricted antigens (CD19 to CD22). A number of other antibodies were also used (anti-CD10, OKT9, OKT10, MY7, MY9, OKM1, OKM5, antiplatelet glycoprotein Ib, IIb/IIIa, anti-MHC class II molecules DR, DP, DQ) (23). The specificity of these antibodies has been widely published. A cell population was

considered positive if >30% of the neoplastic cells reacted with the mAb. The surface phenotypes of the 22 cases of B-lineage ALL studied are summarized in Table I. The B-restricted antigen CD19 was expressed in all but one case, and the antigen CD10 (cALLA) was found in 18 cases. In none of the cases studied did the blasts express the T-restricted or associated antigens CD1, CD3, CD4, CD7, and CD8. As described further on, rearrangement of the H chains of Ig was observed in all of the cases studied. The literature contains a considerable body of evidence for assigning this type of proliferation to the B cell line (8). To describe the stages of early pre-B differentiation, we used the model of Nadler et al. (8). We also studied the phenotypes and genotypes of 19 chronic B cell malignancies (10 chronic lymphocytic leukemias (CLL) and 9 hairy cell leukemias (HCL)). The B nature of these proliferations was certified by demonstrating the expression of a single isotype of Ig L chain. In the rare cases where the cells did not express surface Ig, the presence of the molecules CD19, CD20, and CD21 was considered as sufficient evidence for assigning these proliferations to the B cell line. To compare the patterns of rearrangement of TRG $\gamma$  in B cell malignancies and in T cells, T cells isolated by E rosetting from the blood of healthy donors and blast cells from 19 patients with acute T cell malignancies were also studied. The surface phenotype of these malignant cells was representative of the different stages of the thymic maturation. B-restricted antigens were not detected in any of the cases, nor were myeloid antigens (anti-MY9), whereas all the cases tested expressed the pan-T molecule CD7 (24). The CD3 molecule was expressed in 5 of 18 cases of T-ALL, 4 of which also expressed either CD4 or CD8 (Reinherz's stage III [25]). Of the CD3<sup>-</sup> cases, seven expressed at least one of the common thymocyte antigens (stage II) and seven only pan-T markers (CD5 and CD7) (stage I or I/II). As controls, nine typical cases of AML (21) were also studied.

*DNA and RNA Extraction, Southern and Northern Blots.* These procedures were done according to the usual methods. High molecular weight DNA was digested by the restriction endonucleases Bam HI, Hind III, and Eco RI under conditions recommended by the manufacturer (Promega Biotec, Madison, WI). The restriction fragments were separated in 0.6% agarose gel by electrophoresis at 40 V for 24 h and were transferred onto a hybridization membrane (Hybond-N; Amersham Corp., Arlington Heights, IL). The prehybridization and hybridization were performed at 65°C in 6 $\times$  SSC (1 $\times$  SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7), 0.2% SDS, 10% (wt/vol) dextran sulfate, and sonicated denatured salmon sperm DNA at 200  $\mu$ g/ml. The filters were washed for 15 min in 2 $\times$  SSC, 0.2% SDS at room temperature; for 15 min in the same solution at 65°C; 30 min in 0.2 $\times$  SSC, 0.2% SDS; and then 30 min in 0.1 $\times$  SSC, 0.2% SDS at 65°C. The blots were autoradiographed for 2–4 d. The extraction of total mRNA was performed by the guanidium-cesium chloride method (26). The cell pellet was lysed by vortexing into 6 M guanidium isothiocyanate, 5 mM sodium citrate, 0.1 M 2-ME, and 0.5% sarkosyl. The lysate was then centrifuged over a gradient of 5.7 M cesium chloride at 77,000 *g* for 16 h at 20°C. The RNA was precipitated in 70% ethanol and resuspended in diethylpyrocarbonate-treated water. 15  $\mu$ g of total RNA were electrophoresed through a 1.2% agarose-formaldehyde gel and transferred to nitrocellulose or nylon membrane. The prehybridization and hybridization were performed in 4 $\times$  SSPE (1 $\times$  SSPE is 0.18 M NaCl, 1 mM EDTA, 10 mM NaH<sub>2</sub>PO<sub>4</sub>), 0.2% SDS, 0.1% Nappi, and 5  $\mu$ g/ml of heparin. The filters were washed for 1 h in 1 $\times$  SSPE, 0.1% SDS and 0.5 $\times$  SSPE and 0.5 $\times$  SSPE, 0.1% SDS at 68°C.

*DNA Probes.* The TCR $\beta$  probe is a cDNA fragment specific for the constant region of the  $\beta$  chain of the TCR (a Bgl II/Bgl II fragment of the 4D1 cDNA [27]) kindly provided by J. L. Strominger, Cambridge, MA. The J $\gamma$  probe is a subcloned Eco RI–Hind III fragment of the MH60 J $\gamma$  clone, kindly provided by T. H. Rabbitts, Cambridge, United Kingdom (16). The C $\gamma$  probe is the 0.7 kb Bam HI fragment of the PHT  $\gamma$ 9 cDNA encoding for a VJC gene, kindly provided by J. L. Strominger (18). The entire PHT  $\gamma$ 9 cDNA probe was used to detect the TRG $\gamma$  transcripts. C $\mu$  is the 1.2 kb genomic probe C75p1.2 (28), kindly provided by T. H. Rabbitts. Probes were <sup>32</sup>P-labeled by nick translation or by oligolabeling (multiprime labeling kit from Amersham Corp.). TRG $\gamma$  rearrangement was interpreted assuming a loop excision mechanism and using the

TABLE I  
Surface Phenotype and Genotype of Malignant Cells from Patients with B-Lineage ALL

Patient	Blast cells %	Surface phenotype*							Stage <sup>‡</sup>	Status of IgH genes <sup>‡</sup>	T $\gamma$ genes			TCR $\beta$ genes			mRNA expression
		DR	CD10	CD19	CD20	CD22 <sup>‡</sup>	CD22 <sup>‡</sup>	E			Rearrangement		Status	Rearrangement		mRNA expression	
											Type <sup>§</sup>	H		$\beta$ 1	$\beta$ 2		
1	97	+	-	+	-	+	+	II	R/R	G	R	1	a	a	ND	ND	
2	96	+	-	+	-	+	+	II	R/R	R	R	1	a	a	ND	ND	
3	89	+	-	+	-	+	+	II	ND	G	G				ND	ND	
4	98	+	-	+	-	+	+	I-II	R/R	G	G				ND	ND	
5	86	+	+	+	-	+	+	III	R/D	R	R	1, JP?	c	c	+	+	
6	90	+	+	+	-	+	+	III	R/R	R	R	3	c	c	+	+	
7	86	+	+	+	+	+	+	III-IV	D/R	R	R	1	d	d	ND	ND	
8	73	+	+	+	+	+	+	III-IV	G/R	R	R	3	e	h	+/-	+	
9	80	+	+	+	-	+	+	III	R/R	R	R	1	c	c	ND	ND	
10	90	+	+	+	-	+	+	III	R/R	R	R	JP, JP			+	+	
11	95	+	+	+	-	+	+	III	R/R	G, R	R	2	c, e	b, e	ND	ND	
12	96	+	+	+	-	+	+	III	D/D	R	R	1	e	b	+	+	
13	88	+	+	+	-	+	+	III	ND	R	R	1	e	b	ND	ND	
14	93	+	+	+	-	+	+	III-IV	R/R	R	R	1	e	h	ND	ND	
15	87	+	+	+	-	+	+	III	G/R	R	R	JP			+	+	
16	89	+	+	+	-	+	+	III-IV	D/R	R	R	D	i	c	+	+	
17	95	+	+	+	-	+	+	III	D/D	R	R	2	c, f	i, e	+	+	
18	80	+	+	+	-	+	+	III	G/R	R	R	1	e	h	+	+	
19	80	+	+	+	-	+	+	III	D/R	R	R	2 or 5	c, c	e, e	+	+	
20	99	+	+	+	-	+	+	III	R/R	G	G				+	+	
21	95	+	+	+	-	+	+	IV	D/R	R	R	5	d, f	i, d	ND	ND	
22	98	+	+	+	-	+	+	Ind	R/R	R	R	J	a	a	+	+	

\* Negative results and those obtained with OKT10, OKT9, and anti-MHC DQ and DP molecule antibodies are not shown. +/-, 20-30% positive cells.  
<sup>‡</sup> The presence of intracytoplasmic CD22 antigen (21) is indicated in parentheses.  
<sup>§</sup> According to Nadler et al. (8).  
<sup>¶</sup> Using a *C $\mu$*  probe and DNA digested with Bam HI. R, rearranged; G, germ line; D, deleted.  
<sup>†</sup> Defined in Materials and Methods. JP defined in reference 17. H and E, rearranged bands observed respectively with Hind III and Eco RI (see text).

following nomenclature: type 1, monoallelic J $\gamma$ 1 rearrangement; type 2, biallelic J $\gamma$ 1; type 3, monoallelic J $\gamma$ 2; type 4, biallelic J $\gamma$ 2; type 5, J $\gamma$ 1 on one allele and J $\gamma$ 2 on the other. Representative cases are shown in Fig. 2. TCR $\beta$  chain gene rearrangement was interpreted as previously described (14).

## Results

*TRG $\gamma$  Rearrangement and Expression in B-lineage ALL.* As shown in Table I, a germline configuration was found only in four cases. Of the remaining 18 cases, 10 (cases 2, 7–10, 12–14, 18, and 22) showed one rearranged band and two germline bands in DNA digested by the three restriction enzymes, which is compatible with monoallelic J $\gamma$ 1 or J $\gamma$ 2 rearrangement by loop excision (cases 12 and 22 are shown in Fig. 3). Except in cases 6 and 8, the intensity of the bands would be more suggestive of J $\gamma$ 1 rearrangement (a representative case is shown in Fig. 2, group 1). The noninvolvement of the J $\gamma$ 2 region in these eight cases was also supported by the presence of two germline bands of almost equal intensity when the same filters were hybridized with the C $\gamma$  probe (e.g., Fig. 4, case 10). In Fig. 4, cases 6 and 8, the bands Hind III of 12.5 kb and Eco RI of 4.3 kb that correspond to the C $\gamma$ 1 region appeared less intense, and the overall results are more consistent with monoallelic J $\gamma$ 2 rearrangement (type 3). In Fig. 3, cases 17, 19, and 21, the presence of two rearranged bands and the disappearance of the germline band corresponding to the J $\gamma$ 1 region are compatible with biallelic rearrangement of J $\gamma$ 1 (type 2) or with J $\gamma$ 1 rearrangement on one allele and J $\gamma$ 2 on the other (type 5). Comparing band intensities for filters hybridized with the J $\gamma$  probe and the C $\gamma$  probe (Fig. 4) suggests type 5 rearrangement for case 21 and type 2 for case 17. For case 19 it is not easy to distinguish between type 2 and 5. For the 5 remaining cases with rearranged bands, interpretation is more difficult, but in two instances the findings are fully consistent with the involvement of the JP region (Fig. 5). As summarized in Table I, the J $\gamma$ 1 region was involved in 12 of 18 cases of B-lineage ALL in which TRG $\gamma$  were rearranged. This contrasts with the data obtained in the 19 T-ALL cases studied as controls. TRG $\gamma$  rearrangement was observed in 18 cases. The J $\gamma$ 1 region was never involved and in most cases; the patterns observed were consistent with a biallelic involvement of the J $\gamma$ 2 region (Fig. 1 and Table II).

To analyze the rearrangement of the different V $\gamma$  segments in malignant B cell and T cell precursors and in peripheral T cells, the sizes of the rearranged bands observed using the J $\gamma$  probe were compared. Six faint rearranged bands were observed in the DNA of polyclonal normal T cells upon digestion with Hind III or Eco RI enzymes. With one exception (band *Eb-Hf*), all the bands seen in polyclonal T lymphocytes were also found in B-lineage ALL (Fig. 3) and in T-lineage ALL (not shown). Moreover, in B-lineage ALL, two additional bands were seen, Eco RI of 4.7 kb (*Ei*) and Hind III of 11.5 kb (*Hi*). The *Eh* band found in B-lineage ALL but not in polyclonal T cells was also found in T-lineage ALL. Excepting band *He*, which may be associated with band *Eb* or *EH*, a one-to-one relation can be established between the bands seen after digestion of DNA by Hind III and by Eco RI. For the bands *Ha* to *He* this relation is identical to the one found in T-ALL. Bands *Hc-Ee* were seen in 6 of 18 rearranged B-lineage ALL cases. The patterns for certain cases were identical (Table I).

In 13 cases, TRG $\gamma$  mRNA expression was investigated. With the techniques

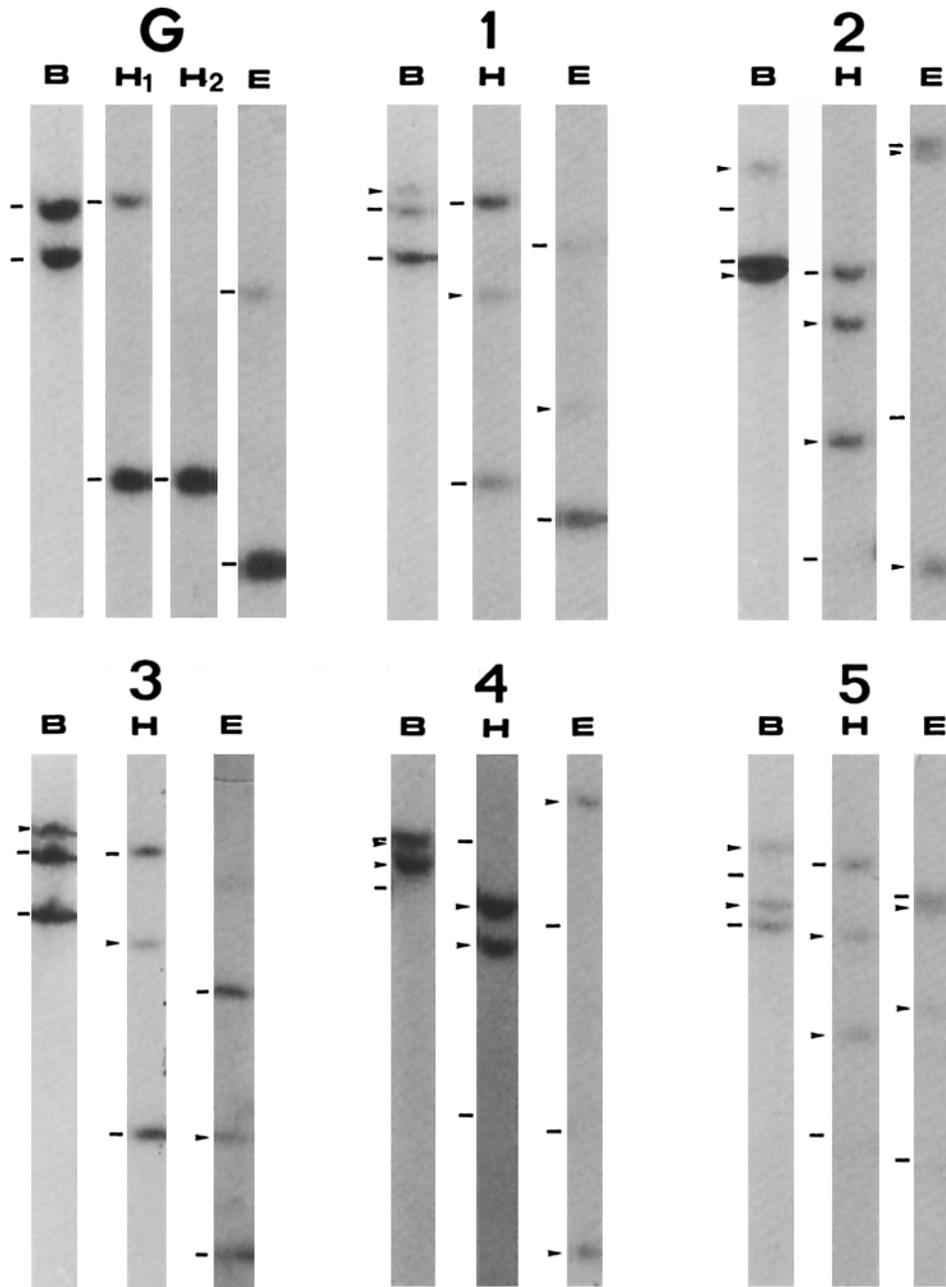


FIGURE 2. Germline configuration and rearrangement of  $J\gamma$  genes. (G) DNA from normal granulocytes digested by Bam HI (B), Hind III (H), and Eco RI (E). (H<sub>1</sub> and H<sub>2</sub>) The polymorphism of the Hind III site 5' upstream to the  $J\gamma 2$  segment (see Fig. 1A). (Groups 1-5) The different typical patterns of  $J\gamma$  rearrangement. (1) Monoallelic  $J\gamma 1$  rearrangement; (2) biallelic  $J\gamma 1$  rearrangement; (3) monoallelic  $J\gamma 2$  rearrangement. The faint 5.2 kb Eco RI band in this case probably represents an Eco RI site relatively resistant to normal digestion; (4) biallelic  $J\gamma 2$  rearrangement; (5)  $J\gamma 1$  rearrangement on one allele and  $J\gamma 2$  on the other.

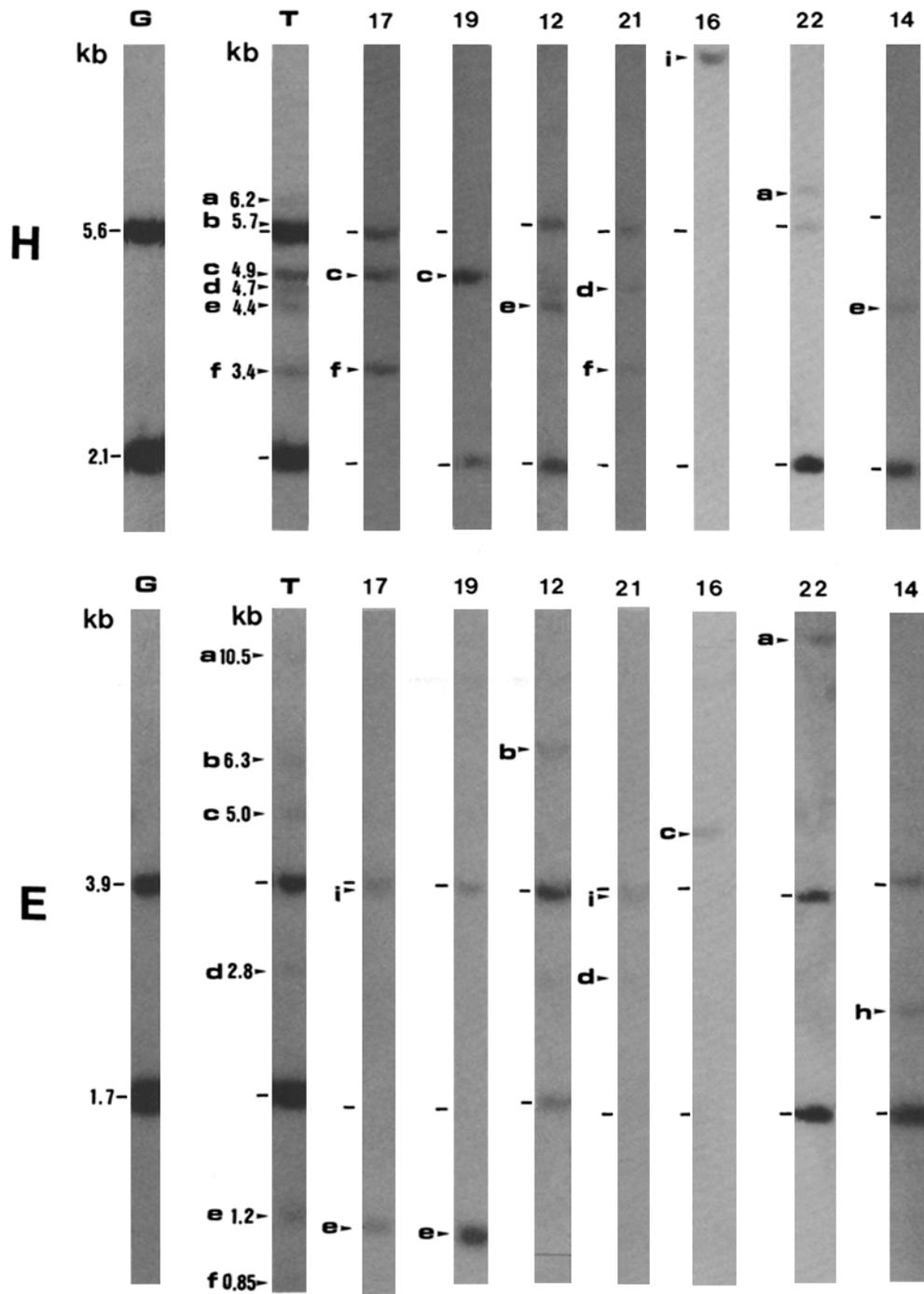


FIGURE 3. Germline and rearranged bands in DNA digested by Hind III (*H*) and Eco RI (*E*) and hybridized with the  $J\gamma$  probe. (*G*) Germline pattern observed in DNA from normal granulocytes. (*T*) Normal polyclonal circulating T cells (*E*+ fraction). The other lanes are the samples from B-lineage ALL patients with the same case numbers as in Table I. *Ha* to *Hf* and *Ea* to *Ef* are the rearranged bands observed in the DNA of polyclonal T lymphocytes digested with Hind III and Eco RI, respectively. The rearranged bands *Hi* and *Ei* are observed in neither the polyclonal T cells nor the T-ALL cells and correspond to bands *Ec* and *Hf*, respectively.

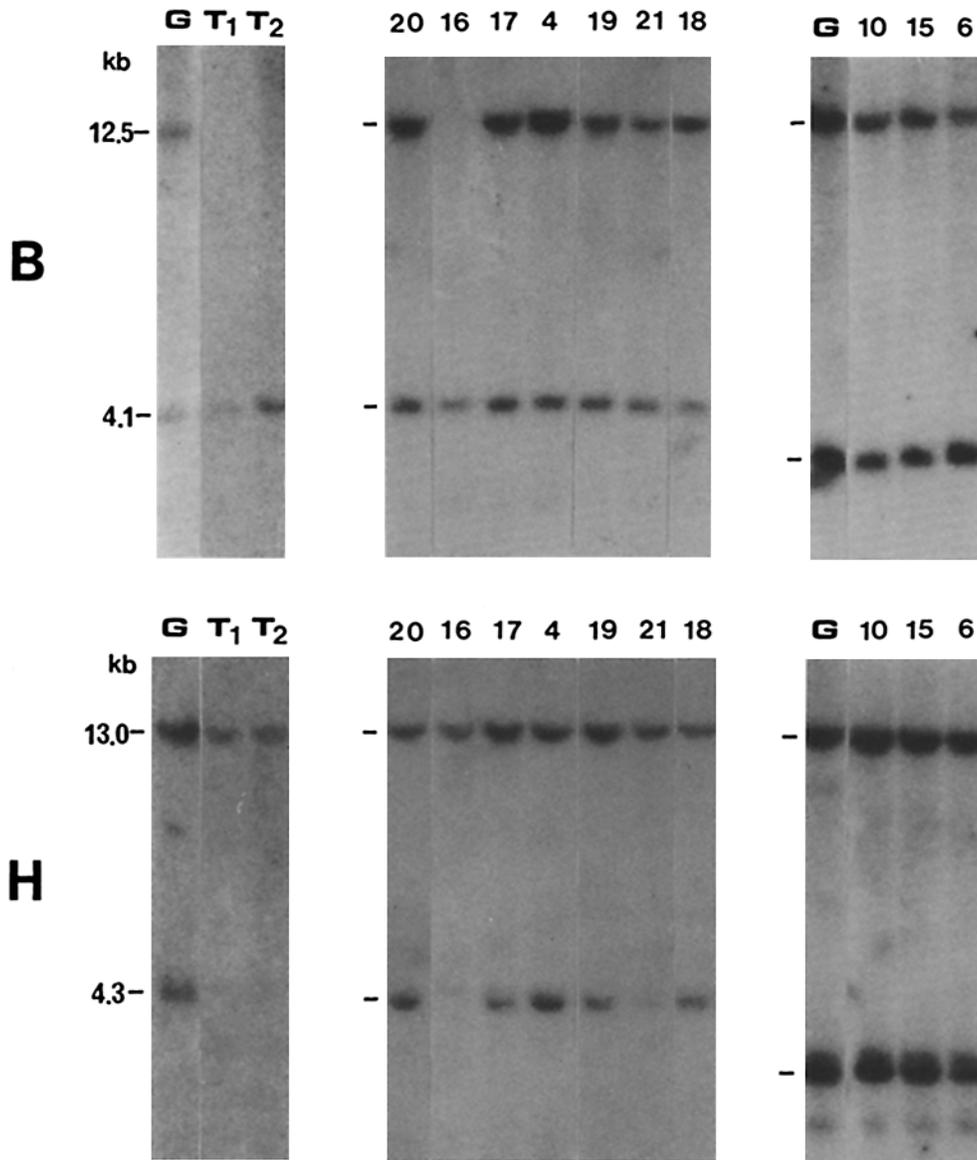


FIGURE 4. DNA digested with Bam HI (B) and Hind III (H) and hybridized with the C $\gamma$  probe. (G) Germline pattern. As controls, T-ALL CEM cell line (T<sub>1</sub>) and one T-ALL case (T<sub>2</sub>) show the deletion of the C $\gamma$ 1 segment corresponding to a biallelic J $\gamma$ 2 rearrangement (type 4). The other lanes are the samples from B-lineage ALL patients with the same case numbers as in Table I. In case 16, a deletion of the C $\gamma$ 1 region is observed. The decreased intensity of the C $\gamma$ 2 band suggests a deletion of C $\gamma$ 2 region on one allele, in line with the result obtained using the J $\gamma$  probe (see Fig. 3 and text). In cases 21 and 6, the decreased intensity of the C $\gamma$ 1 band as compared with the germline pattern of each experience (case 20 and the nearer G lane) corresponds to a J $\gamma$ 2 rearrangement on one allele. In these two cases the findings obtained with the J $\gamma$  probe are consistent, respectively, with type 5 and 3 rearrangements. The absence of diminution of intensity of C $\gamma$ 1 band in the cases in which rearranged bands are seen after J $\gamma$  probing (cases 10, 15, 17, 18, and 19) is in favor of J $\gamma$ 1 or JP involvement. In cases 4 and 20 a germline pattern was also found using the C $\gamma$  probe. The same result was obtained using the J $\gamma$  probe (data not shown).

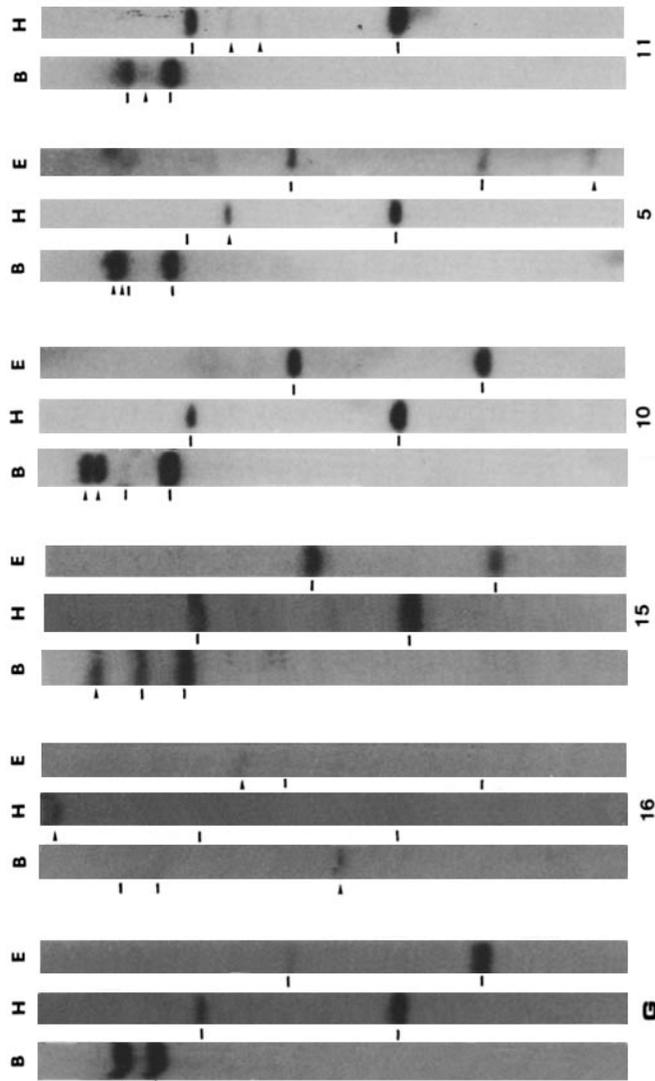


FIGURE 5. Atypical patterns of  $J\gamma$  rearrangement in ALL of B cell lineage. (G) Germline band of DNA extracted from normal granulocytes and digested with Bam HI (B), Hind III (H), and Eco RI (E). In case 16 the DNA digested by the three enzymes exhibited a single rearranged band, with disappearance of the germline bands. Furthermore, the intensity of the rearranged band was weak. The disappearance of the band corresponding to the  $C\gamma 1$  region and reduction of the intensity of the band corresponding to the  $C\gamma 2$  region was also noted after hybridizing the filter with the  $C\gamma$  probe (Fig. 2). To check that the quantity of DNA transferred was comparable to that of other DNA present on the same filter, the filter was hybridized with the  $TCR\beta$  probe. A rearranged pattern was observed with bands of comparable intensity to other cases (Fig. 7). These findings suggest rearrangement of the  $J\gamma 2$  region, along with deletion on the other chromosome of a region including at least  $J\gamma 1$  and  $J\gamma 2$ . Since genes  $T\gamma$  and  $TCR\beta$  are respectively located at 7p15 and 7q35 (references 29, 30), these results imply that this probable deletion does not involve the whole of one of the two chromosomes 7. In cases 15 and 10, one and two rearranged bands, respectively, were found only in DNA digested by the Bam HI enzyme, whereas a germline pattern was seen after digestion by Hind III and Eco RI. This could reflect monoallelic

(case 15) or biallelic (case 10) rearrangement involving the JP region, which has recently been described by Lefranc et al. (17). Since JP rearrangement by loop excision does not affect the  $J\gamma 1$  region (see Fig. 1), the persistence of two germline bands of equal intensity in DNA hybridized with the  $C\gamma$  probe (Fig. 4) is consistent with this hypothesis. In case 11, besides the germline bands, two rearranged bands of weaker intensity than expected for type 2 rearrangement were visible after digestion of the DNA by Hind III and Eco RI. One possibility is that this pattern is due to contamination by polyclonal T cells. This hypothesis does not seem very likely given: (a) the percentage of blasts and CD10+ cells in the sample, (b) the relatively strong band intensity, and (c) the presence of only two bands instead of the five or six expected. Another possibility is that this pattern is due to the mixture of blasts with T $\gamma$  genes in germinal configuration (for region  $J\gamma 1$  and  $J\gamma 2$ ), and a subclone with rearranged T $\gamma$  genes. In this case there is no evidence of a subpopulation with a distinctive membrane phenotype (data not shown). In case 5 the observed pattern is very difficult to account for by rearrangement by loop excision without a probe that recognizes the JP segment; it may be a case of JP rearrangement on one allele and  $J\gamma 1$  on the other.

TABLE II  
*T Cell and B-Lineage ALL: Comparison of TRG $\gamma$  Rearrangement Patterns*

Cell line	Number of cases with rearrangement	Type*					Others
		1	2	3	4	5	
T-ALL	18/19	0	0	0	14	0	4
B-lineage ALL	18/22	8	1	2	0	1	6

\* Type 1, monoallelic J $\gamma$ 1 rearrangement; type 2, biallelic J $\gamma$ 1; type 3, monoallelic J $\gamma$ 2; type 4, biallelic J $\gamma$ 2; type 5, J $\gamma$ 1 on one allele and J $\gamma$ 2 on the other.

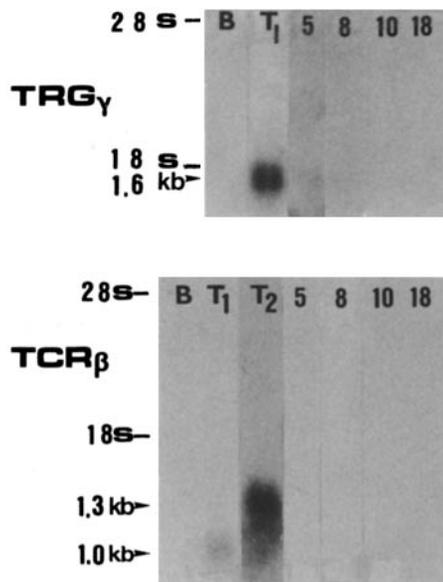


FIGURE 6. Northern blots hybridized with J $\gamma$  and TCR $\beta$  probes. In addition to negative (EBV cell lines) and positive (T-ALL case) controls, two cases with weak TRG $\gamma$  mRNA transcription (cases 5 and 8) and two cases with no detectable TRG $\gamma$  mRNA are shown. The other cases did not express detectable TRG $\gamma$  mRNA and are not shown. Transcription of TCR $\beta$  mRNA was never found in B-lineage ALL cases.

used, no TRG $\gamma$  mRNA transcript was detected in 11 of the cases. Weak expression was seen in cases 5 and 8 in the form of 1.6 kb transcripts (Fig. 6). Case 5 showed stronger expression than case 8, but the expression was nevertheless far weaker than in T cell ALL used as a control.

*Correlation between TRG $\gamma$  Gene Rearrangement, Surface Phenotype, and Rearrangement of the Ig H Chains and TCR $\beta$  Chain Genes.* In ALL, fewer stage II cases (DR+CD19+) showed rearrangement of TRG $\gamma$  genes (1 of 4 cases) than did stage III cases (DR+CD19+CD10+; 1 of 16 cases). No relation was noted between TRG $\gamma$  gene rearrangement and the pattern of rearrangement of the Ig H chain genes. In 9 of 22 cases, rearrangement of the TCR $\beta$  chain genes was observed. The presence of such rearrangement was not correlated with the immunological phenotype. In none of the rearranged cases did the J $\beta$ 1 region seem to be involved. Monoallelic rearrangement of the J $\beta$ 2 region was found in 5 of the 9 rearranged cases, and biallelic J $\beta$ 2 involvement in the other cases (Fig. 7). In all of the rearranged cases one or two C $\beta$ 1 regions were deleted, according to whether the J $\beta$ 2 rearrangement was mono- or biallelic. In none of the cases could TCR $\beta$  mRNA expression be detected (Fig. 6). It is interesting to note that

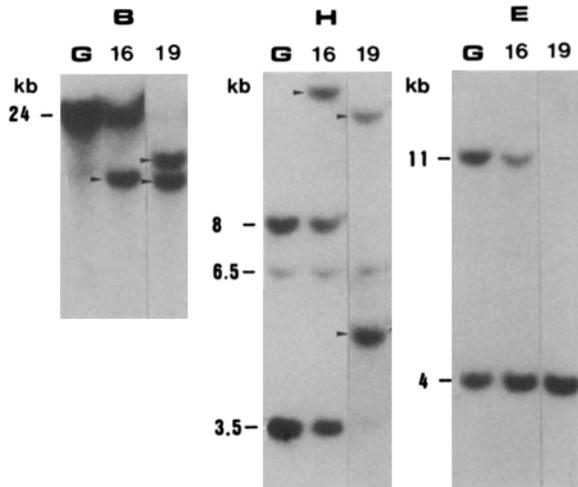


FIGURE 7.  $TCR\beta$  rearrangement in B-lineage ALL cases. (G) Germline pattern from normal granulocytes. Cases 16 and 19 are representative cases of monoallelic (case 16) and biallelic (case 19)  $J\beta 2$  rearrangement.

in all the cases (except one) with the  $TRG\gamma$  in germinal configuration the  $TCR\beta$  genes were not rearranged.

*$TRG\gamma$  and  $TCR\beta$  Chain Rearrangement in AML and Chronic B Cell Proliferations.* In none of the 9 AML cases studied was  $TRG\gamma$  and  $TCR\beta$  chain gene rearrangement found. The  $TRG\gamma$  were seen in germline configuration in all 19 cases of B lymphoid malignancies. The  $TCR\beta$  chain genes were rearranged in one B-CLL.

### Discussion

In this study we have demonstrated  $TRG\gamma$  rearrangement in 18 of 22 cases of ALL expressing surface antigens characteristic of early pre-B maturation. Rearrangement was more frequent in stage III cases  $DR+CD19+CD10+$  according to the model of Nadler et al. (8), than in  $DR+CD19+$  cases considered less mature.

It is unlikely that these results are due to contamination of the samples by polyclonal T cells. We have shown that as for thymocytes (17), five or six rearranged bands are detectable by Southern blotting in the DNA of polyclonal T cells after digestion by Hind III and Eco RI and hybridization with a  $J\gamma$  probe (Fig. 3). In the rearranged B-lineage ALL cases, only one or two rearranged bands were found. Furthermore, the intensity of the rearranged bands was not consistent with the contamination level that would ensue from the percentage of blasts (Table I) and of cells of T phenotype present in the samples (data not shown). Thus it is very likely that the rearranged bands detected correspond to the DNA of malignant cells. Moreover, these findings cannot be ascribed to selection of atypical cases; the blast phenotypes and the presence of rearrangement of the H chain Ig genes are in agreement with the data published in the literature (4, 8).

There were differences between the rearrangements of the  $TRG\gamma$  genes seen in B and T cell proliferations. We have observed that in T-ALL the rearrangements are often biallelic and involve the  $J\gamma 2$  region (type 4 rearrangement). In our series of T-ALLs we did not find rearrangement of the  $J\gamma 1$  segment.

However, in B-lineage ALL, TRG $\gamma$  rearrangement involved the J $\gamma$ 1 regions in 12 of 18 rearranged cases, whereas the J $\gamma$ 2 region was involved in only 4 instances. Furthermore, the J $\gamma$ 1 rearrangement was monoallelic in 8 cases. The frequent involvement of the J $\gamma$ 2 region in the rearrangement of TRG $\gamma$  in T cells had already been noted, one of the proposed explanations being that successive rearrangements involve first J $\gamma$ 1 then J $\gamma$ 2 during somatic development (31). If this hypothesis is correct, then our results, as shown in the discussion below, suggest that J $\gamma$ 1 rearrangement may occur in very immature cells that still have both B and T potentiality.

As in T-ALL, the rearranged bands seen in B-lineage ALL were often of the same size in different patients. Thus bands *Hc-Ee* (Fig. 3) were present in 6 of 18 rearranged cases. Most of the rearranged bands seen in B-lineage ALL were also detected in T-ALLs, polyclonal T lymphocytes, and thymocytes (17), which would suggest that the same V $\gamma$  genes are implicated in the rearrangements of TRG $\gamma$ . In 11 of the 13 B-lineage ALL cases investigated for mRNA expression, no TRG $\gamma$  transcript was detectable. In two cases, weak TRG $\gamma$  mRNA (1.6 kb) expression was noted. It is difficult to affirm that these mRNAs reflect TRG $\gamma$  gene transcription by neoplastic cells or that they are due to contaminating mature T cells. It should nevertheless be noted that in these cases, and particularly in case 5, the contamination was not higher than in other cases in which no transcription was detected. Moreover, as shown by studies in mice (32), TRG $\gamma$  mRNA expression in mature T cells is probably weak.

9 of the 22 B-lineage ALL cases studied exhibited TCR $\beta$  chain gene rearrangement that was not correlated with surface phenotype. The rate of rearrangement seemed slightly higher than the rate reported in a recent study (11 of 39 cases [7]). In our series we found only the J $\beta$ 2 region to be involved. This agrees with the data inferred from the report of Tawa et al. (reference 7, Table II). In all of our rearranged cases one or two C $\beta$ 1 regions were deleted, according to whether the J $\beta$ 2 rearrangement was mono- or biallelic. This would suggest that rearrangement of the J $\beta$ 2 regions is not merely D $\beta$ 2-J $\beta$ 2 joining. However, the involvement of the V $\beta$  genes in the rearrangement of TCR $\beta$  chain genes remains to be demonstrated. In our series, TCR $\beta$  chain gene rearrangement was not accompanied by any TCR $\beta$  mRNA expression.

The significance of TRG $\gamma$  and TCR $\beta$  chain gene rearrangement in B-lineage ALL remains obscure. Since no expression of these genes was detected in most cases, it is possible that mRNA transcription is inhibited by a regulation mechanism. It may be that at an early stage of lymphoid maturation, rearrangement of TRG $\gamma$ , TCR $\beta$  chain genes and Ig H chain genes can occur, randomly or otherwise. This hypothesis is supported at least in part, since for the genes of the H chain of Ig and of the TCR $\beta$  chain gene, recent experiments have shown that a pre-B cell can rearrange an exogenous TCR $\beta$  D $\beta$ 1-J $\beta$ 1 fragment introduced by transfection (33). The recombination mechanism could be regulated by controlling the accessibility to non-cell line-specific recombinase usable both by B and T cells (33). Only in the cases where the rearrangement is productive does the cell inhibit rearrangement of other Ig-like genes by a mechanism analogous to allelic exclusion (34) and is thus directed towards a B or T lineage according to the gene involved.

An important point is whether TRG $\gamma$  and TCR $\beta$  chain gene rearrangements can occur in normal counterparts of B leukemic cells, as has been suggested for rearrangement and expression of Ig H chain genes in mouse T cells (35, 36). Assuming that the rate of rearrangement seen in leukemic cells reflects the rate in normal cells, it is intriguing to find little TCR $\beta$  chain gene rearrangement and especially no TRG $\gamma$  rearrangement in chronic B cell proliferations. It is conceivable that these rearrangements occur more easily in ALL cells. Alternatively, it may be that these rearrangements occur in normal B cell precursors but that these cells are then unable to achieve normal differentiation and/or attain sufficient expansion. Recently, it was found (37, 38) that the mechanism of translocation may be close to the mechanism of physiological rearrangement. It is possible that the transcriptional status of Ig-like genes, at particular stages of lymphoid maturation, makes them more accessible to recombinase action (33) and more exposed to some chromosomal aberrations. This could explain the frequent localization of breakpoints at the chromosome bands corresponding to the loci of Ig and TCR genes (39–42), and supplies a model, possibly of general interest, for explaining the specificity of certain chromosomal translocations with respect to the lineage involved in lymphoid malignancies and to the stage of differentiation of neoplastic cells.

### Summary

T cell rearranging gene  $\gamma$  (TRG $\gamma$ ) and T cell antigen receptor  $\beta$  (TCR $\beta$ ) chain gene rearrangement and transcription were studied in a series of patients with B-lineage acute lymphoblastic leukemia (ALL), in which the Ig H chain genes are rearranged and the surface phenotype reproduces the stages of normal pre-B maturation. For comparison, polyclonal T cells from peripheral blood of healthy donors and blast cells from 19 cases of T lineage ALL were also studied. In this study we demonstrate the presence of a clonal rearrangement of the TRG $\gamma$  in 18 of the 22 B-lineage ALL cases and establish that this rearrangement, which generally involves the J $\gamma$ 1 region, is often monoallelic and appears different from the biallelic J $\gamma$ 2 rearrangement frequently seen in T-cell ALLs. In 9 of 22 cases, we found rearrangement of the genes of the TCR $\beta$  chain, which never involved the J $\beta$ 1 region. Conversely, the TRG $\gamma$  were seen in germline configuration in all 19 cases of B chronic lymphoid malignancies. In none of the 9 AML cases studied was TRG $\gamma$  and TCR $\beta$  chain gene rearrangement found. The TCR $\beta$  chain genes were rearranged in one B cell chronic lymphocytic leukemia (CLL). We also show that in B-lineage ALL, the cells probably use the same V $\gamma$  genes for TRG $\gamma$  rearrangements as the malignant cells in T-ALL and the polyclonal T cells. In none of the 13 B-lineage ALL cases investigated by Northern analysis was TCR $\beta$  mRNA expression detected, whereas a weak expression of TRG $\gamma$  transcripts was found in two of these cases. The correlations between surface phenotype, rearrangement of TRG $\gamma$ , TCR $\beta$ , and Ig H chain genes were analyzed. The significance of rearrangement of TRG $\gamma$  and TCR $\beta$  chain genes in B or pre-B cells is also discussed.

We are indebted to T. H. Rabbitts and J. L. Strominger who have supplied the DNA probes. We thank Dr. M. P. Lefranc for critical reading of this manuscript.

*Received for publication 10 September 1986 and in revised form 8 December 1986.*

### References

1. Arnold, A., J. Cossman, A. Bakhsi, E. S. Jaffe, T. A. Waldmann, and S. J. Korsmeyer. 1983. Immunoglobulin gene rearrangements as unique clonal markers in human lymphoid neoplasms. *N. Eng. J. Med.* 309:1593.
2. Flug, F., P. G. Pellicci, F. Bonetti, D. M. Knowles, II, and R. Dalla-Favera. 1985. T-cell receptor gene rearrangements as markers of lineage and clonality in T-cell neoplasms. *Proc. Natl. Acad. Sci. USA.* 82:3460.
3. Waldman, T. A., M. M. Davis, K. F. Bongiovanni, and S. J. Korsmeyer. 1985. Rearrangement of genes for the antigen receptor on T-cells as markers of lineage and clonality in human lymphoid neoplasms. *N. Eng. J. Med.* 313:776.
4. Korsmeyer, S. J., A. Arnold, A. Bakhsi, U. Siebfnlst, P. A. Hieter, S. O. Sharrow, T. W. Lebien, J. H. Kebsey, D. G. Poplack, P. Leder, and T. A. Waldmann. 1983. Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemias of T-cell and B-cell precursor origins. *J. Clin. Invest.* 71:301.
5. Ha, K., M. Minden, N. Hozuni, and E. W. Gelfand. 1984. Immunoglobulin chain gene rearrangement in a patient with T-cell acute lymphoblastic leukemia. *J. Clin. Invest.* 73:1232.
6. Pelicci, P. G., D. M. Knowles, II, and R. Dalla-Favera. 1985. Lymphoid tumors displaying rearrangements of both immunoglobulin and T cell receptor genes. *J. Exp. Med.* 162:1015.
7. Tawa, A., N. Hozumi, M. Minden, T. W. Mak, and E. W. Gelfand. 1985. Rearrangement of the T-cell receptor  $\beta$ -chain gene in non-T cell non-B cell acute lymphoblastic leukemia of childhood. *N. Eng. J. Med.* 313:1033.
8. Nadler, L. M., S. J. Korsmeyer, K. C. Anderson, A. W. Boyd, B. Slaughenhoupt, E. Park, J. Jensen, F. Coral, R. J. Mayer, S. E. Sallan, J. Ritz, and S. F. Schlossman. 1984. B-cell origin of non-T cell acute lymphoblastic leukemia: a model for discrete stages of neoplastic and normal pre-B cell differentiation. *J. Clin. Invest.* 74:332.
9. Rovigatti, U., J. Mirro, G. Kitchingman, G. Dahl, J. Ochs, S. Murphy, and S. Stass. 1984. Heavy chain immunoglobulin gene rearrangement in acute nonlymphocytic leukemia. *Blood.* 63:1023.
10. Ha, K., M. Minden, N. Hozumiand, and E. W. Gelfand. 1984. Immunoglobulin gene rearrangement in acute myelogenous leukemia. *Cancer Res.* 44:4658.
11. Palumbo, A., J. Minowada, J. Erikson, C. M. Croce, and G. Rovera. 1984. Lineage infidelity of a human myelogenous leukemic cell line. *Blood.* 64:1059.
12. Cheng, G. Y., M. D. Minden, B. Toyonaga, T. W. Mak, and E. A. McCulloch. 1986. T cell receptor and immunoglobulin gene rearrangements in acute myeloblastic leukemia. *J. Exp. Med.* 163:414.
13. Korsmeyer, S., P. A. Hieter, J. V. Ravetch, D. G. Poplack, T. A. Waldmann, and P. Leder. 1981. Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B cells. *Proc. Natl. Acad. Sci. USA.* 78:7096.
14. Sangster, R. N., J. Minowada, N. Suciufoca, M. Minden, and T. W. Mak. 1986. Rearrangement and expression of the  $\alpha$ ,  $\beta$ , and  $\gamma$  chain T cell receptor genes in human thymic leukemic cells and functional T-cells. *J. Exp. Med.* 163:1491.
15. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. *Nature (Lond.).* 312:36.

16. Lefranc, M. P., and T. H. Rabbitts. 1985. Two tandemly organized human genes encoding the T-cell constant region sequences show multiple rearrangement in different T-cell types. *Nature (Lond.)*. 316:464.
17. Lefranc, M. P., A. Forster, R. Baer, M. A. Stinson, and T. H. Rabbitts. 1986. Diversity and rearrangement of the human T cell rearranging gamma genes: nine germ-line variable genes belonging to two subgroups. *Cell*. 45:237.
18. Quertermous, T., C. Murre, D. Dialynas, A. D. Duby, J. L. Strominger, T. A. Waldman, and J. G. Seidman. 1986. Human T-cell  $\gamma$  chain genes: organization, diversity and rearrangement. *Science (Wash. DC)*. 231:252.
19. Lefranc, M. P., A. Forster, and T. H. Rabbitts. 1986. Rearrangement of two distinct T-cell gamma chain variable region genes in human DNA. *Nature (Lond.)*. 319:420.
20. Haars, R., W. Kronenberg, M. Gallatin, I. L. Weissman, F. L. Owen, and L. Hood. 1986. Rearrangement and expression of T cell antigen receptor and T $\gamma$  genes during thymic development. *J. Exp. Med.* 164:1.
21. Bennett, J. M., D. Catovsky, M. T. Daniel, G. Flandrin, D. A. G. Galton, H. R. Gralnick, and C. Sultan. 1976. Proposals for the classification of acute leukaemias. *Br. J. Haematol.* 33:451.
22. Foon, K. A., and S. F. Todd, III. 1986. Review: immunological classification of leukemia and lymphoma. *Blood*. 68:1.
23. Chen, Z., F. Sigaux, R. Miglierina, F. Valensi, M. T. Daniel, M. H. Ochoa-Noguera, and G. Flandrin. Immunological typing of acute lymphoblastic leukemia: concurrent analysis by flow cytofluorometry and immunocytology. *Leukemia Res.* 10:1411.
24. Pitaluga, S., M. Raffeld, H. Lipford, and J. Cossman. 1986. 3A1 (CD7) expression precedes T $\beta$  gene rearrangement in precursor T (lymphoblastic) neoplasms. *Blood*. 68:134.
25. Reinherz, E. L., P. C. Kung, G. Goldstein, R. H. Levey, and S. F. Schlossman. 1980. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proc. Natl. Acad. Sci. USA*. 77:1588.
26. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*. 18:5294.
27. Jones, N., J. Leiden, D. Dialynas, J. Fraser, M. Clabby, T. Kishimoto, and P. Strominger. 1984. Partial primary structure of the alpha and beta chains of human tumor T-cell receptors. *Science (Wash. DC)*. 227:311.
28. Rabbitts, T. H., A. Forster, and C. Milstein. 1981. Human immunoglobulin heavy chain genes: evolutionary comparison of C $\mu$ , C $\delta$ , and C $\gamma$  genes, and associated switch sequences. *Nucleic Acids Res.* 9:4509.
29. Murre, C., T. A. Waldmann, T. B. Shows, and J. G. Seidman. 1985. Human  $\gamma$ -chain genes are rearranged in leukemic T cells and map to the short arm of chromosome 7. *Nature (Lond.)*. 316:549.
30. Le Beau, M. M., M. O. Diaz, J. D. Rowley, and T. W. Mak. 1985. Chromosomal localization of the human T cell receptor  $\beta$ -chain genes (letter). *Cell*. 41:335.
31. Quertermous, T., W. Strauss, C. Murre, D. P. Dialynas, J. L. Strominger, and J. G. Seidman. 1986. Human T-cell  $\gamma$  genes contain N segments and have marked junctional variability. *Nature (Lond.)*. 322:184.
32. Raulet, D. H., R. D. Garman, H. Saito, and S. Tonegawa. 1985. Developmental regulation of T-cell receptor gene expression. *Nature (Lond.)*. 314:103.
33. Yancopoulos, G. D., T. K. Blackwell, H. Suh, L. Hood, and F. W. Alt. 1986. Introduced T-cell receptor variable region gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. *Cell*. 44:251.
34. Richie, K. A., R. L. Brinster, and U. Storb. 1984. Allelic exclusion and control of

- endogenous immunoglobulin gene rearrangement in  $\kappa$  transgenic mice. *Nature (Lond.)*. 312:517.
35. Kemp, D. J., A. Wilson, A. W. Harris, and K. Shortman. 1980. The immunoglobulin constant region gene is expressed in mouse thymocytes. *Nature (Lond.)*. 286:168.
  36. Kurosawa, Y., V. H. Boehner, W. Haas, H. Sakano, A. Trauneker, and S. Tonegawa. 1981. Identification of D segments of immunoglobulin heavy chain genes and their rearrangement in T lymphocytes. *Nature (Lond.)*. 290:565.
  37. Tsujimoto, Y., E. Jaffe, J. Cossman, J. Gorham, P. C. Nowell, and C. M. Croce. 1985. Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation. *Nature (Lond.)*. 315:340.
  38. Tsujimoto, Y., J. Gorham, J. Cossman, E. Jaffe, and C. M. Croce. 1985. The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. *Science (Wash. DC)*. 229:1390.
  39. Klein, G. 1983. Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. *Cell*. 32:311.
  40. Croce, C., M. Isobe, A. Palumbo, J. Puck, J. Ming, D. Tweardy, J. Erikson, and M. Davis. 1985. Gene for alpha-chain of human T-cell receptor: location on chromosome 14 region involved in T-cell neoplasms. *Science (Wash. DC)*. 227:1044.
  41. Rabbitts, T. H., M. P. Lefranc, M. A. Stinson, J. E. Sims, J. Schroder, M. Steinmetz, N. L. Spurr, E. Solomon, and P. N. Goodfellow. 1985. The chromosomal location of T-cell receptor genes and a T-cell rearranging gene: possible correlation with specific translocations in human T cell leukemia. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:1461.
  42. Baer, R., K. C. Chen, S. D. Smith, and T. H. Rabbitts. 1985. Fusion of immunoglobulin variable gene and a T-cell receptor constant gene in the chromosome 14 inversion associated with T cell tumors. *Cell*. 43:705.